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DEPOSIT, AQUATIC FATE AND SHORT-TERM EFFECTS OF TRICHLORFON AFTER AERIAL FORESTRY APPLICATIONS IN NEWFOUNDLAND

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DEPOSIT, AQUATIC FATE AND SHORT-TERM EFFECTS OF TRICHLORFON AFTER AERIAL FORESTRY APPLICATIONS IN NEWFOUNDLAND

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ABSTRACT

In an effort to control damage to conifer forests in western Newfoundland by the balsam fir sawfly (*Neodiprion abietis*) an aerial spray program using Dylox® (a.i. trichlorfon) was conducted in July 1998. To protect aquatic organisms known to be susceptible to trichlorfon, a 200 m buffer zone was established around watercourses. That buffer zone was established using a combination of predictive drift modeling (AgDrift) and professional judgment. A study was undertaken to determine the effectiveness of the buffers by measuring deposit on collectors, aquatic contamination and effects on invertebrates in operational spray blocks after spray. Two ponds and two streams were monitored where Dylox® (PCP # 16,387) was applied at a rate of 750 g ai/ha by two M-18 fixed wing aircraft flying at 185 km/h, 10 m above the canopy, employing Micronair AU5000 rotary atomizers calibrated to deliver a mean droplet diameter of 120 microns. The aircraft were directed by a Bell 206B helicopter.

At the time of application, it was observed that one pond/stream system received a buffer of only between 30 to 60 m. In that block, deposit on collectors at the watercourse edge ranged from concentrations equivalent to 0.44 to 50.07 g/ha of trichlorfon. The second block, which was observed as receiving the 200 m protection, was not treated entirely on the same day (applications to separate portions of the block were separated by 24 h), and deposits on collectors deployed at the edge of that pond/stream system ranged from 0.78 to 18.76 g/ha trichlorfon.

The highest concentration of trichlorfon was 1124.7 µg/L in pond water in the block which received the smaller buffer. While those concentrations were near the range of the 96 h LC_{50} for rainbow trout of 330 - 2500 µg/L (Howe *et al.* 1994) and over 200 times greater than the 96 h LC_{50} for stonefly at 5.3 µg/L (Woodward and Mauck 1980), the duration of elevated concentrations were much shorter than those LC_{50} exposure times, ranging from 0.5 to 14.5 h post-treatment. However, some water samples from time periods up to 14 h post-spray exceeded invertebrate (*Daphnia carinata*) LC_{50} values for comparable exposure times (i.e. 3 - 6 h LC_{50}). Water samples taken immediately after spraying were not toxic according to a Microtox assay. All samples up to 5 h post-treatment from the poorly buffered block immobilized *Daphnia magna* and lethal effects

were observed for 4 out of 7 samples, during 48 h exposures. Phytoplankton from the pond with the smaller buffer were reduced in 24 of 30 species present (90% total number reduction after 24 h, 65% reduction after 48 h); however, it did not have as marked a reduction in numbers compared to the pond which received the 200m buffer. Aquatic macroinvertebrates in the poorly buffered stream declined after treatment with a 2/3 reduction in the numbers of individuals post-24 h.

In the block which received the 200 m buffer, the highest trichlorfon concentration (900 µg/L) measured was in a mid-depth sample of pond water taken at 3 h post-spray. Water samples taken immediately after spraying were not toxic according to a Microtox assay. Samples taken at 2 h and 3 h post-treatment at the buffered block immobilized *Daphnia magna* during 48 h exposures. After treatment, phytoplankton numbers were dramatically reduced in 24 of 26 species present (99 % total number reduction) in pond samples. Aquatic macroinvertebrates in the stream declined slightly for 5 of the 7 genera present in pre-spray samples; however, total numbers of invertebrates were greater after spray (48 h) than prior to spray.

The results and observations indicated that a 200 m watercourse buffer zone for the application of Dylox® at 750 g a.i./ha, is inadequate to prevent deposition of trichlorfon at concentrations that pose a risk to aquatic organisms. That risk is further increased when the buffers are not implemented. The risks to fish cannot be as easily estimated; however, trichlorfon concentrations in water may present some direct toxicological threat and indirect effects such as stress from food reduction cannot be eliminated.

RÉSUMÉ

Pour lutter contre les dommages causés aux forêts de conifères de l'ouest de Terre-Neuve par le diprion du sapin (*Neodiprion abietis*), on a appliqué un programme de pulvérisation aérienne utilisant le Dylox^{MC} (trichlorfon comme matière active) en juillet 1998. Afin de protéger les organismes aquatiques sensibles au trichlorfon, on a établi une zone tampon de 200 m autour des masses d'eau. On a établi cette zone tampon en combinant un modèle de prévision de la dérive (AgDrift) et le jugement professionnel. On a entrepris une étude pour déterminer l'efficacité des zones tampons par la mesure du dépôt sur des collecteurs, de la contamination aquatique et des effets sur les invertébrés dans les blocs de pulvérisation opérationnels après la pulvérisation. On s'est penché sur deux étangs et deux cours d'eau où du Dylox^{MC} (numéro d'enregistrement du produit antiparasitaire : 16,387) a été appliqué à un taux de 750 g m.a./ha par deux avions M18 volant à 185 km/h à 10 m au-dessus du couvert forestier, et utilisant des pulvérisateurs centrifuges Micronair AU5000 calibrés pour projeter des gouttelettes d'un diamètre moyen de 120 microns. Les avions étaient dirigés par un hélicoptères Bell 206B.

Au moment de l'application, on a observé qu'un système étang-cours d'eau a bénéficié d'une zone tampon de seulement 30 à 60 m. Dans ce bloc, les concentrations du dépôt sur les collecteurs en bordure des masses d'eau équivalaient à 0,44 à 50,07 g de trichlorfon /ha. Le second bloc, où la zone tampon a bien été de 200 m, n'a pas été traité entièrement le même jour (les applications sur les diverses portions du bloc ont été réalisées à des intervalles de 24 h), et les concentrations des dépôts sur les collecteurs installés en bordure de ce système étang-cours d'eau équivalaient à 0,78 à 18,76 g de trichlorfon/ha.

La plus forte concentration de trichlorfon, 1124,7 µg/L, a été observée dans les eaux d'un étang du bloc où la zone tampon était réduite. Bien que ces concentrations approchaient de la fourchette de la CL_{50}-96 h pour la truite arc-en-ciel (330-2 500 µg/L, Howe *et al.*, 1994) et étaient plus de 200 fois supérieures à la CL_{50}-96 h pour les plécoptères (5,3 µg/L, Woodward et Mauck, 1980), elles sont demeurées élevées seulement 0,5 à 14,5 h après le traitement, soit beaucoup moins longtemps que les 96 h de ces CL_{50}. Cependant, les concentrations dans des échantillons d'eau mesurées jusqu'à 14 heures après pulvérisation excédaient les CL_{50} pour un invertébré (*Daphnia carinata*) pour des périodes d'exposition

comparables (CL_{50} sur 3 à 6 heures). Les échantillons d'eau prélevés immédiatement après pulvérisation n'étaient pas toxiques selon un essai Microtox. Tous les échantillons prélevés jusqu'à 5 h après le traitement dans le bloc à zone tampon réduite ont immobilisé *Daphnia magna*, et des effets létaux ont été observés chez 4 échantillons sur 7 après expositions de 48 h. Les effectifs du phytoplancton de l'étang à zone tampon réduite ont chuté chez 24 des 30 espèces présentes (réduction des effectifs totaux de 90 % après 24 h, réduction de 65 % après 48 h); cependant, la réduction des effectifs y a été moins marquée que dans l'étang dont la zone tampon était de 200 m. Les effectifs des macroinvertébrés aquatiques dans le cours d'eau à zone tampon réduite avaient chuté des 2/3 24 h après le traitement.

Dans le bloc à zone tampon de 200 m, la plus forte concentration de trichlorfon (900 µg/L) a été mesurée dans un échantillon prélevé au milieu de la colonne d'eau d'un étang 3 h après le traitement. Les échantillons d'eau prélevés immédiatement après pulvérisation n'étaient pas toxiques selon un essai Microtox. Les échantillons prélevés 2 et 3 h après le traitement dans le bloc à zone tampon de 200 m ont immobilisé *Daphnia magna* lors d'expositions de 48 h. Après le traitement, les effectifs de phytoplancton ont chuté radicalement chez 24 des 26 espèces présentes (réduction des effectifs totaux de 99 %) dans les échantillons prélevés dans des étangs. Les effectifs de macroinvertébrés aquatiques de cours d'eau ont diminué légèrement chez 5 des 7 genres présents dans les échantillons recueillis avant pulvérisation; cependant, les effectifs totaux des invertébrés étaient plus élevés après (48 h) qu'avant la pulvérisation.

Les résultats de cette étude ont montré qu'une zone tampon de 200 m autour des masses d'eau pour l'application du Dylox[MC] à 750 g m.a./ha est insuffisante pour prévenir le dépôt de trichlorfon à des concentrations qui présentent des risques pour les invertébrés aquatiques. Ces risques se trouvent accrus si l'on n'établit pas de zone tampon. Les risques pour les poissons sont difficiles à estimer; cependant, les concentrations de trichlorfon dans l'eau peuvent avoir des effets toxicologiques directs, et on ne peut éliminer leurs effets indirects, comme le stress qu'elles peuvent imposer sur le réseau trophique par réduction des ressources alimentaires.

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1.0 INTRODUCTION

An aerial pesticide spray program was proposed for late July and early August 1998, by the Newfoundland Department of Forest Resources & Agrifoods to combat the balsam fir sawfly (*Neodiprion abietis*) in western Newfoundland. A preliminary risk assessment indicated the potential for significant aquatic deposit and effects on aquatic organisms if watercourses were not protected from direct spray. A dispersion model (AgDrift) was used to predict off-target deposit under operational conditions and that deposit when combined with the biological endpoints of *Salmo clarki* (cutthroat trout) 96 h LC_{50} of 375 µg/L (Woodward and Mauck 1980), *Daphnia carinata* (water flea) 48 h LC_{50} of 0.75 µg/L (Nishiuchi 1979), and *Pteronarcella badia* (stonefly) 96 h LC_{50} of 5.3 µg/L (Woodward and Mauck 1980), indicated that watercourse buffer zones of at least 200 m would be required to protect sensitive aquatic invertebrates. A 200 m buffer zone was therefore made a condition of the provincial authorization permit.

The provincial operator's permit specified buffering watercourses identified on a 1:50,000 topographical map within the treatment area. As well, any lentic water bodies that were visible from the air during pretreatment reconnaissance flights were required by the federal research permit to be protected by a 100 m buffer zone. The operational dosage of 750 g of trichlorfon (the active ingredient in Dylox® 420 PCP# 16,387)/ha was applied at a height of 10 m above the canopy by two M-18 fixed wing aircraft employing AU5000 rotating atomizers to forestry blocks in western Newfoundland at a speed of 185 km/h. This study was undertaken to determine the aquatic contamination and effects on invertebrates of the operational spray program employing the 200 m watercourse buffer zone.

2.0 SAMPLING SITE LOCATIONS

One pond and one stream were selected for sampling in each of the two treatment blocks (213 and 215) (Figure 1) situated east of Stephenville, Newfoundland in the area of the Long Range Mountains (48°36' 58" N, 58°01' 02" W and 48°34' 58" N, 58°02' 57" W respectively.) Accessible smaller pond/stream systems within spray blocks were selected

for monitoring since they represent the worst case situation because dilution was expected to be a smaller factor in such systems.

2.1 Block 213

On July 27, 1998 at approximately 7:10 am, 6 flight lines (70 ha) of Block 213 (total 186 ha) were sprayed. At the time of spraying, the wind speed was recorded as SW (magnetic) at 2-3 km/h according to the chief pointer. The remainder of Block 213 was treated on the morning of July 28 (3 flight lines, 24 ha), wind speed SSW at 5 km/h. Therefore, post-spray samples for Block 213 were taken twice (24 h apart) and are referred to as Block 213 and Block 213B samples. The pond in Block 213 (200 m X 150 m) was surrounded by balsam fir and alder to its edges except for the west side, where the stream entered the pond. The first 200 m upstream from the pond had little canopy cover and the remainder had approximately 50% canopy cover. The depth of the pond was 1-1.5 m with a pH of approximately 6.8. The pH measurements were taken in Environment Canada's Environmental Conservation Branch laboratory within 48 h of sample collection using a Metrohm E588 meter. The stream was a primary stream with a width of 0.3 m and a depth of 2-15 cm. The water temperature was approximately 17°C, according to long-term water quality data collected in nearby rivers by the Nfld Department of Environment and Labour (Nfld Department of Environment and Labour 1993). It appeared from pointer aircraft observation that Block 213 and 213B received the intended 200 m buffer around the pond and stream.

2.2 Block 215

On July 27, 1998 at approximately 6:50 am, Block 215 (148 ha) was sprayed with Dylox® along 13 spray lines. The pond in Block 215 was smaller measuring approximately 30 m X 10 m with a depth of 1-1.5 m. The pond had a pH of approximately 5.9, as measured by the Environmental Conservation laboratory, and was vegetated to its edges with alder and balsam fir, except on the west side where the stream entered under a gravel road, where vegetation was sparse alder growth. The first 40 m of the primary stream was without canopy cover. The remaining length of the stream had approximately 85% cover. The stream was approximately 0.3 m wide and 2-15 cm deep. The water temperature was approximately 17°C (Nfld Department of Environment and Labour

1993). Records of the chief pointer for the spray program indicate that this stream and pond received a buffer of between 30 m and 60 m.

3.0 METHODS

3.1 Surface deposition

Spray deposit collectors consisted of rectangular solvent rinsed 20 X 25 cm teflon coated glass fiber filters attached to 10 precleaned 20 X 25 cm stainless steel plates, mounted on steel rods. Those collectors were randomly deployed over the surface of the pond. Five to seven stainless steel plates and filters were also placed along the stream margins for a distance of 150 m in Block 213 and 50 m in Block 215. Thirty minutes after the spray, the filters were carefully removed from the plates, folded and placed into 250 mL solvent washed glass bottles. The exposed filters, along with 4 filter blanks, were sent to the Department of Fisheries and Oceans laboratory located in St. John's, Newfoundland for analysis of trichlorfon and its degradation product dichlorvos (Appendix A). Samples were maintained at 4°C until analysis was conducted, approximately 6 months after collection.

3.2 Concentration of trichlorfon / dichlorvos in water samples

Water samples were taken prior to treatment and up to 14.5 h post treatment for analysis of trichlorfon and dichlorvos content. Water samples were collected in the incoming streams (8-11 samples/block) as well as in the ponds (12-15 samples/block) from both blocks. Surface samples were collected in both the streams and the ponds and mid-depth samples were collected in the ponds in 1 L amber glass bottles using a weighted bottle sampler. Approximately 50 mL of dichloromethane was added to the 1 L amber bottles and the bottles were subsequently shaken for approximately 3 min. The samples were kept on ice in coolers and shipped to the Department of Fisheries and Oceans laboratory in St. John's, Newfoundland. One dichloromethane blank was also sent to the laboratory for analysis (Appendix A). The limits of quantification for trichlorfon and dichlorvos were 1.50 µg/L and 0.25 µg/L respectively. Analysis occurred approximately 6 months after collection.

3.3 Toxicity

Individual water samples for bioassay were taken prior to treatment and up to 5 hours post-spray in 1 litre glass jars with food-grade polyethylene film lined lids. The water samples were kept on ice during shipment to the Environment Canada Laboratory in Moncton, New Brunswick for bioassay within 48 h of sampling. The toxicity tests were conducted using the freshwater crustacean, *Daphnia magna*, according to the Environment Canada standard protocol (Environment Canada 1990), incorporating the amendments from May, 1996. The samples were also analyzed for toxicity to the luminescent bacterium *Vibrio fischeri* using the Microtox analyzer 100 % test protocol according to Environment Canada (1992) and Microbics (1992).

3.4 Phytoplankton and zooplankton

A 60 cm diameter plankton net (0.5 mm mesh) with a 100 mL sample container was lowered to the bottom of each pond, at a depth of approximately 1 m. Duplicate plankton samples were collected prior to treatment and 48 h after the first treatment. At Block 215, 48 h post-spray samples were collected. To immobilize and allow settling of the plankton, 2-propanol was added to the sample. Additional 2-propanol was added when the container was decanted, after the organisms had settled to the bottom of the jar. Ten subsamples were sent to Dr. Ellen Kenchington, Dalhousie University, Halifax, NS for phytoplankton species identification and enumeration. The samples were prepared using the filter-transfer-freeze technique (Hewes and Holm-Hansen 1983). Ten samples were subsampled for zooplankton identification and enumeration by Dr. Christine Campbell, Memorial University, Sir Wilfred Grenfell College, Corner Brook, Nfld. Samples were filtered through 80 µm mesh then made up to 20 mL with 95% ethanol. In most cases, the entire 20 mL were then examined for zooplankton. For samples with a large number of individuals (some samples with lots of copepods) only 10 mL were examined. Samples were examined at 160 to 250 X magnification, in a plexiglass counting wheel, under a Leitz dissecting microscope.

3.5 Aquatic macroinvertebrates

A Surber sampler (0.1 m^2) was used to collect benthic invertebrates prior to treatment and 24 h after application in Block 213. Block 215 was sampled prior to treatment, 24 h and 48 h after application along the stream. Six samples were collected from each stream, at riffle areas, at each sample time. To preserve the samples, 2-propanol was added to each container.

Drift nets (20 cm square, 0.5 mm mesh) were positioned at two locations separated by a distance of approximately 10 m in each stream to collect drifting macroinvertebrates. When collecting, net openings were in contact with the bottom of the stream and allowed to collect for 15 min intervals for each sample event. The nets were subsequently removed and the contents were carefully washed into 500 mL glass jars with stream water. To preserve the samples, 2-propanol was added to the jars. Pre-treatment sampling was conducted one week prior to spray at 9:30 p.m., 10:30 p.m. and 11:30 p.m. and at 3:30 am and 4:30 am. Evening drift samples at 7:45 p.m. and 8:45 p.m. were also collected two days pre-treatment. Following a morning spray, samples were collected at 30 minutes and then at one hour intervals until 4 h post-treatment in Block 215. The night immediately following the spray event, samples were collected approximately at the same time of day as the pre-treatment samples. For Block 213, the drift sampling occurred after the second application at the above stated time intervals, and the samples are referred to as Block 213B samples. The macroinvertebrates were identified and enumerated by Dr. Ken Neil, Kentville, Nova Scotia.

4.0 RESULTS and DISCUSSION

4.1 Surface deposition

The data from Block 213 is presented as two data sets due to the split application on this Block. The July 27 data indicated relatively consistent deposit of trichlorfon in both the pond and stream border samples with values ranging from 0.64 to 9.14 g/ha (Table 1). Dichlorvos residues on deposit collectors ranged between 0.48 and 3.41g/ha, which when combined with the trichlorfon residues gives an average total deposit of 0.10 to 1.22% of the emitted application rate. On July 28, the trichlorfon residue values were greater,

ranging from 0.57 to 18.76 g/ha. Dichlorvos values ranged from 0.93 to 3.88 g/ha, and when combined with the trichlorfon yields a total deposit of 0.12 to 2.5% of the emitted application rate.

Trichlorfon residues were measured on more of the collectors on July 27 than on July 28, despite the fact that there was no spraying near the pond. All flights on the first day were downslope below the elevation of the pond and the wind was parallel to the flight lines at SW 2-3 km/h. That wind direction would not have been expected to produce drift to the pond. There were higher dichlorvos values on July 28 and sporadic trichlorfon deposits when the actual insecticide formulation releases were upwind and upslope of the study pond.

The measured deposit of trichlorfon on collectors at Block 215, which received the buffer of 30 - 60 m, ranged from 0.36 to 45.54 g/ha. Positive detections for dichlorvos ranged from 1.15 to 4.53 g/ha along the margins of both the pond and the stream in this block. The combined residue value (trichlorfon and dichlorvos) is approximately 0.06 to 6.68% of the emitted application rate.

Since it has been previously shown that deposit within spray blocks receiving similar types of application can range from 30 to 50% of the intended dosage, it is apparent that the buffer zones did reduce the potential of deposit on the watercourses by as much as 90% (Mickle 1999).

4.2 Concentration of trichlorfon / dichlorvos in water samples

4.2.1 Ponds

The analytical results of the water samples taken from the ponds between 30 minutes and 14.5 hours post-spray indicate that pesticide residue was present in concentrations up to 1124.7 µg/L. Due to the fact that the buffer was less in Block 215, it is not surprising that the highest recorded concentration for trichlorfon was that watercourse at 3 h post-spray (Table 2). While those concentrations were near the range of the 96 h LC_{50} for rainbow trout of 330 - 2500 µg/L (Howe *et al.*, 1994) and 200 times greater than the 96 h LC_{50} for stonefly at 5.3 µg/L (Woodward and Mauck 1980), the duration of elevated

concentrations were shorter than standard bioassay exposure times (96 h), ranging from only 0.5 to 14.5 h post-treatment (Table 3). Trichlorfon toxicity tests, with shorter exposure periods using *Daphnia carinata,* produced LC_{50} values ranging from 88 µg/L for 1 h to 0.75µg/L for 48 h (Nishiuchi 1979) (Figures 2a, 3a & 4a). Overall, 57% of all the pond water samples analyzed exceeded the 1 h LC_{50} for *Daphnia carinata* and the maximum exceedance was approximately 12.8 times that LC_{50}. For Block 215, where the buffer was not observed, 80% of the pond water samples had concentrations which exceeded the 1 h LC_{50} for *Daphnia carinata*. In Block 213B this value was 71% and for Block 213 it was 18%.

All of the pond water samples in the poorly buffered pond had positive detections for trichlorfon, dichlorvos or both, while 82% - 93% of the samples from the 200 m buffered pond had positive pesticide detections. Since trichlorfon degrades to dichlorvos, which is more toxic to aquatic organisms (EPA 1987), the presence of both trichlorfon and dichlorvos in water bodies could produce an additive toxic effect on aquatic organisms. The conversion to dichlorvos is reasonably rapid with the average half-life of trichlorfon being 30-40 h in a lake at pH ≤ 6 and 5°C (Hazardous Substances Data Bank 1998). The degradation rate increases with an increase in pH. Studies have shown that trichlorfon hydrolyzed to dichlorvos in water with half-lives of about 588 h, 67 h, and 22 h at respective pHs of 6, 7, and 8 (Hazardous Substances Data Bank 1998). The temperatures in the systems monitored were probably somewhat higher (est. 17°C) and conversion to dichlorvos would be expected to proceed more quickly; however, data were not available to estimate how much that conversion rate would change.

The dichlorvos concentrations detected in the pond samples ranged from 1.8 µg/L to 40.7 µg/L, with the highest concentration detected in Block 215. Since the times of aquatic sampling were shorter than that required to see significant hydrolysis in pond/stream after release, it is possible that the hydrolysis occurred as a result of pesticide mixing prior to application or hydrolysis after sampling. None of the water samples analyzed had dichlorvos concentrations above the rainbow trout 96 h LC_{50}, which is 100 µg/L (EPA 1987); however, 69% of the pond water samples had dichlorvos concentrations which were above the 96 h LC50 for stonefly, 0.10 µg/L (Hazardous Substances Data Bank

1998). Since the limit of quantification for dichlorvos was 0.25 µg/L, all of the positive detections for dichlorvos were above the 0.10 µg/L 96 h LC_{50} value for stonefly. Block 213B had the highest occurrence of dichlorvos detections at 79%. Block 213 had the next highest occurrence at 73% and Block 215 had 50%. Dichlorvos half-lives in lakes and rivers are reported to be approximately 4 days (Hazardous Substances Data Bank 1998).

The water samples that were taken mid-depth at 3 h, 13 h or 14.5 h post-spray produced similar concentrations to samples taken at comparable times on the surface of both ponds.

One pre-spray pond water sample from Block 215 had a dichlorvos concentration of 0.57 µg/L. That sample was re-analyzed and no dichlorvos above the level of quantification was measured.

4.2.2 Streams

The results of the analysis of stream water samples taken between 15 minutes post-spray and 14.5 h post-spray indicate that several trichlorfon concentrations were near the range of the 96 h LC_{50} for rainbow trout, 330 - 2500 µg/L (Howe *et al.* 1994). However, those residues persisted in streams for much shorter periods than those used to generate the trout LC_{50}. Overall, 58% of all stream water samples had trichlorfon concentrations which exceeded the 1 h LC50 for *Daphnia carinata,* of 88 µg/L. For the stream in Block 215, which received the 30 - 60 m buffer, 67% of the samples exceeded the 1 h LC50 for *Daphnia carinata* (Figure 2b). For Block 213, which received the 200 m buffer, this value was 57% (Figure 4b) and for Block 213B the value was 50% (Figure 3b). Similar to the pond results, Block 215 had the highest trichlorfon concentration of 965.0 µg/L as well as the highest occurrence of positive detections (89%).

One pre-spray stream water sample from Block 213 had measurable trichlorfon residues (30 µg/L). Re-analysis of the sample indicated a concentration of < 8.0 µg/L. This could indicate a sample contamination problem, since those samples were taken prior to any known spraying in the vicinity; however, their small magnitude should not affect data interpretation.

Not unlike the dichlorvos concentrations in the pond water samples, 63% of all the stream water samples had dichlorvos concentrations which were above the 96 h LC_{50} for stonefly, 0.10 µg/L. In Block 213B, 88% of the stream water samples were above the 96 h LC_{50} for stonefly. For Block 213 and Block 215 the percentage was 71% and 33% respectively.

4.3 Toxicity

None of the samples were toxic to the bacterium, *Vibrio fischeri*, as measured by the Microtox assay. The *Daphnia magna* toxicity test showed no toxicity for Block 213 samples, but significant effects for most of the samples from Block 213B and Block 215 (Table 4).

The samples from Block 213B taken at 0.5 h and 1 h post-spray showed no toxicity to *Daphnia magna*. Samples at 2 and 3 hours post-spray produced complete immobilization of the test organisms. The sample from Block 215 taken pre-spray produced no toxicity to *Daphnia magna;* however, all post-spray samples (taken between 15 min and 5 h post-spray) immobilized all animals. Such results may have ecological significance since immobilization in the wild can affect survival because of increased predator success. The samples between 0.5 h and 3 h produced mortality which ranged from 10 - 80%. The highest mortality rate (80%) was at 1 h post-spray. By comparison, a previous study of the toxicity of fenitrothion contaminated pond water due to forest spraying, Ernst *et al.* (1994) reported a 50% mortality rate for *Daphnia magna* exposed to surface water collected from ponds within 1 h of a direct over-spray of the pond. That level of mortality in *Daphnia* was associated with mortality in rainbow trout (30%) exposed to the same water. Such effects were responsible for the need of implementation of 400 m watercourse buffer zones when spraying fenitrothion.

4.4 Phytoplankton

Pre-treatment phytoplankton samples in Block 213 had a mean total number of individuals of 2578 individuals (Table 5). The 48 hour post-treatment samples (24 h post-spray for Block 213B) had a mean total value of 36 individuals, indicating a 98.6% reduction in total numbers. There was a measured reduction in 24 of 26 species (92 %) during the 24 hour period following the second treatment. Figure 5 illustrates the reduction in total

numbers of individuals post spray as well as reductions in *Navicula* and *Fragilaria* species.

A comparison of total phytoplankton numbers for Block 215, which had a smaller spray buffer than Block 213, did not indicate as substantial a decrease in the numbers present as was observed in Block 213 (Figure 6). Total numbers of phytoplankton were reduced by 90% within 24 h; however, by 48 h there was an apparent increase in numbers to 34% of pre-treatment values. In addition, 24 of the 30 species (80%) that were positively identified had reduced numbers of individuals in the post-treatment samples, up to the 48 h sample period.

While there are no reports in the literature on the effects of trichlorfon on phytoplankton, DeNoyelles *et al.*(1982) reported a 88% reduction of phytoplankton biomass within 10 days of exposure to 500 µg/L atrazine. The biomass recovered to about 94% of the original biomass within approximately 30 days. It could be expected that trichlorfon induced reductions could be of approximately the same duration.

The ecological effects of such population reductions cannot be estimated; however, energy flow through the pond system will be altered due to reduced photosynthetic capability before populations recover.

4.5 Zooplankton

In Block 213, the mean total of zooplankton individuals was 2474/m^3 for the pre-spray samples. The 48 h post-treatment samples (24 h post-spray for the second application) indicated a 48% reduction in total numbers, with reductions for the three genera *Daphnia, Leptodiaptomus* and *Epischura* of 50%, 45% and 58% respectively, of the pre-treatment numbers (Figure 7).

Unlike the phytoplankton reductions, zooplankton were reduced to a greater extent in the pond from Block 215. The 24 h post-treatment samples indicated a 93% reduction in individuals from the pre-treatment samples (Figure 8). Two of the species identified in the pre-treatment samples were absent from the 24 h post-treatment samples. *Chaoborus*

which is a larger zooplankton was absent from both the 24 h and 48 h post-treatment samples. The zooplankton population rebounded to 4.3 times the pre-spray sample numbers within 48 h. Both *Daphnia* and *Leptodiaptomus* numbers increased in the 48 h post-treatment sample by over 5 times from the pre-spray numbers and the recovery following the treatment is 30 to 50 times the 24 h post-treatment numbers. It is possible that the elimination of a predatory species had a releasing effect on the prey species; thereby, causing an increase in total numbers.

4.6 Aquatic macroinvertebrates

Block 215, which received the smaller buffer and had the highest pesticide concentrations, exhibited a marked reduction in numbers of organisms. Total numbers of invertebrates were reduced by 73% within 24 h; however, by 48 h there was an apparent increase in numbers to 55% of pre-treatment values (Fig. 10). In Block 213, there was an apparent increase in total numbers of invertebrates within 48 h of treatment; however, that increase was not significant (Fig. 9).

Changes in the Diptera larvae *Orthocladiinae*, which are burrowers and tube builders, appeared to be responsible for changes in total numbers in both streams (Tables 7 & 8). In Block 215, the 24 h post-treatment Surber results (from 6 Surber samples) indicate a 74 % reduction in the numbers of *Orthocladiinae* individuals. This reduction was not found to be significant at $p=0.05$ (small sample size and variability within samples may have contributed to this result); however, a p-value of 0.054 was calculated using a one-way ANOVA. Individual numbers of *Orthocladiinae* returned to slightly less than half the pre-treatment numbers at 48 h post application (246 - 64 - 108 *Orthocladiinae*). In Block 213, the numbers of *Orthocladiinae* doubled at 48 h post-spray (24 h for Block 213B); however, this was not a significant increase.

In Block 215, the Diptera *Tanypodinae* which are engulfers and piercers, showed a similar reduction and rebound in numbers although with fewer individuals (12 - 7 - 16). Two Ephemeroptera genera, *Baetis* and *Ephemerella* exhibited the same decline and recovery as the Diptera, however those recovery times were small within the time frame. The

stonefly larva *Leuctra*, a clinger, also appears to have been impacted in both Blocks 213 and 215.

No reports of the effects of trichlorfon on lotic benthic invertebrates were found in the literature; however, Grygierek and Wasitewska (1981) indicated marked reductions in pond benthos when trichlorfon was applied resulting in a concentration of 1000 µg/L. Residue levels in pond water from Block 213B were slightly below this level (max. 904 µg/L; 3 h post-spray) while residue levels in pond samples from Block 215 exceeded this level (max. 1125 µg/L; 3 h post-spray).

By comparison, fenitrothion, a previously commonly used forestry insecticide in New Brunswick, resulted in measurable impacts on benthic communities in approximately 15% of the cases where a 'normal' operational dose of 2 X 210 g ai/ha was used (Fairchild *et al.* 1989). Reductions in benthic invertebrates comparable to those measured in Block 215 of this study, have not been generally observed after fenitrothion applications in New Brunswick, unless the dosage rate was up to 2.5 times (560 g ai/ha) that normally used (Penney and MacDonald 1966). There may be some evidence that benthic invertebrate reductions are greater in Newfoundland streams since Coady (1978) measured substantial impacts (70-80% reduction) after spraying of 2 X 210 g ai/ha fenitrothion in Newfoundland.

Although insecticide impacted lotic benthic communities generally return to pre-spray levels within the season, (Fairchild *et al.* 1989) the ecological implications such as overall energy flow and impact on fish populations due to food reductions are difficult to assess.

The number of individuals collected during the aquatic insect drift sampling program was naturally very low, such that the comparisons of the pre and post-treatment samples was not reasonable (Tables 6 & 7). These streams were very small and for that reason did not support large populations of invertebrates. The small number of individuals collected shows a reduction in the total number of individuals post treatment.

5.0 SUMMARY

The sampling indicated that the 200 m watercourse buffer zone, when implemented, was not adequate to prevent deposit and subsequent watercourse contamination, which presented a risk to aquatic organisms. Residue concentrations were near the range of known 96 h rainbow trout LC_{50} values; however, those periods of elevated residue concentrations were much shorter than the LC_{50} exposure times. Large scale fish mortality cannot be predicted from a comparison of residue concentrations with laboratory generated toxicity data; however, it must be recognized that the trichlorfon and dichlorvos residues will probably have an additive effect and additional stressors at the time of application may modify the toxic effect. At all sampling times (up to 14 h post-spray) most water samples exceeded the short-term (3 - 6 h) LC_{50} values for *Daphnia carinata,* a representative aquatic invertebrate. Water samples were found to be toxic to *Daphnia magna* to 3 h post treatment. There were also substantial impacts on pond plankton measured.

In the instance where the watercourse buffer was only 30-60 m, deposits were even greater, resulting in water concentrations which were near the range of the rainbow trout 96 h LC_{50} for short periods of time and exceeding the 96 h LC_{50} for stonefly by 200 times. In those instances there were marked reductions in pond plankton and benthic invertebrates, and all post-spray samples were toxic to *Daphnia magna.*

Overall, the results of this study indicated that a 200 m watercourse buffer zone for the application of Dylox® at 750 g a.i./ha, is inadequate to prevent deposition of trichlorfon at concentrations that pose a risk to aquatic organisms. The risks to fish cannot be as easily estimated, however trichlorfon concentrations in water may present some direct toxicological threat. The indirect effects such as stress from food reduction, are equally difficult to estimate. Spraying with fenitrothion in other areas presented equivocal evidence of fish population reductions (Fairchild *et al.*, 1989); however, invertebrate impacts during those spray programs were generally smaller than those documented in this study and for that reason such an effect cannot be eliminated with these spraying practices. If application parameters remain the same, aquatic buffer zones should be increased for any future Dylox® aerial spray programs.

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APPENDIX A

Modified Analytical Procedure for Analysis of Trichlorfon and Dichlorvos

J. Banoub, Department of Fisheries and Oceans, St. John's Nfld., 1999

The water samples were analyzed by a new method utilizing electrospray tandem mass spectrometry. The water samples were extracted twice with 50 mL of dichloromethane. The organic layers were combined, dried over anhydrous sodium sulfate and evaporated to dryness on a rotary evaporator. The dry residue obtained was dissolved in 1 mL of methanol, filtered and injected in a Micromass Quattro, hexapole-quadrupole-hexapole-quadrupole instrument equipped with an electrospray source operating in the positive ion mode.

The tandem MS/MS method used was Multiple Reaction Monitoring (MRM MS/MS). For trichlorfon, the parent→daughter transitions of the following pairs of ions were monitored: 279.04 →168.61, 279.04→132.80, 280.82→133.11 and 282.97→133.74. The MRM MS/MS method used for analysis of dichlorvos monitored the parent→daughter transitions of the following pairs of ions: 220.89→108.85, 220.89→126.66 and 220.89→108.73. Both MRM MS/MS analyses used a collision energy of 35 eV and a cone fragmentation voltage of 25 volts. The MRM values were plotted on a standard calibration curve with a correlation coefficient of r^2=99.999 and the concentrations were quantified accordingly. The extracts were injected 3 separate times via a loop injector and concentrations were verified using an external standard calibration. The limits of quantification for trichlorfon and dichlorvos were 1.50 µg/L and 0.25 µg/L respectively.

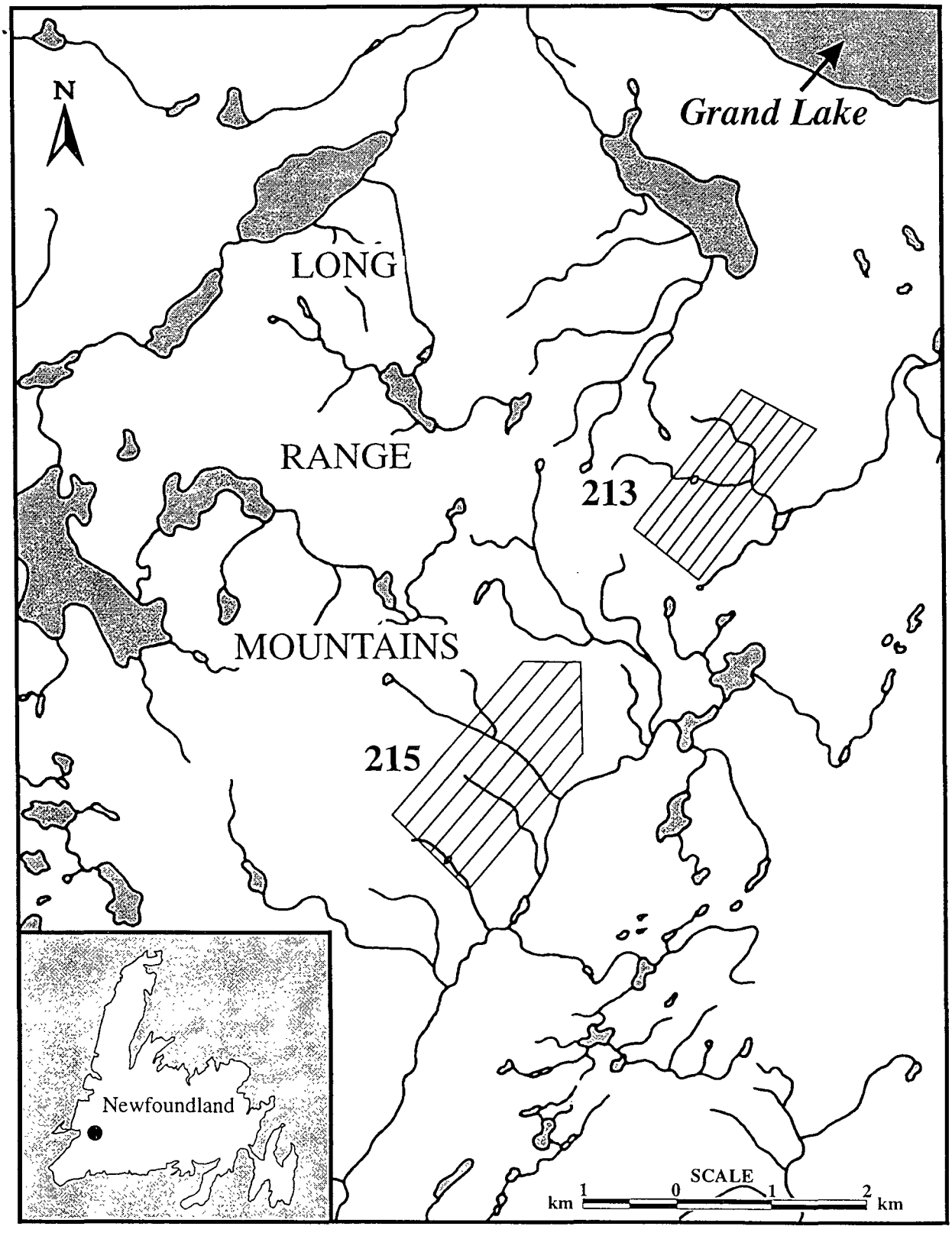


FIGURE 1: Spray Block Locations

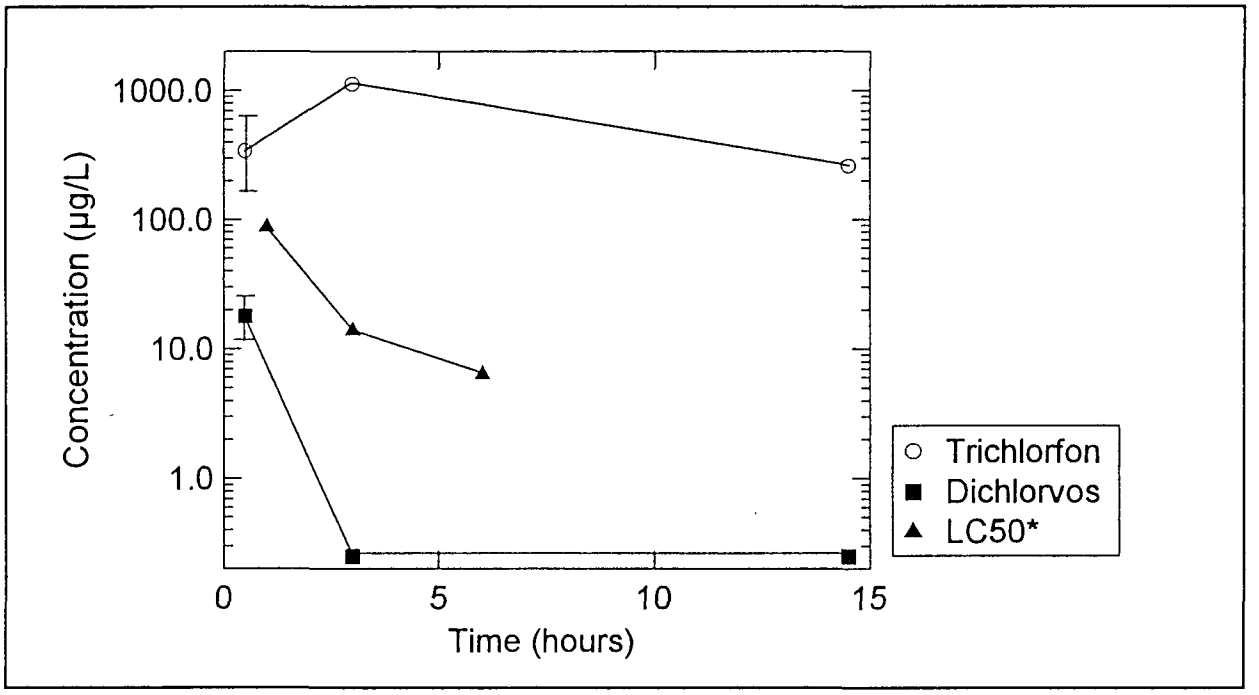


Figure 2a. Pond concentrations (arithmetic mean ± 1 SE) of trichlorfon and dichlorvos following an aerial application of trichlorfon (time=0 hours) in Block 215. Results of trichlorfon LC50 tests (*Daphnia carinata* at 25°C, for 1,3,6 h) are plotted for comparison.

* Nishiuchi, Y. 1979. Acute toxicity of pesticide formulations to *Daphnia carinata*. Suisan Zoshoku 27: 119-124 (in Japanese).

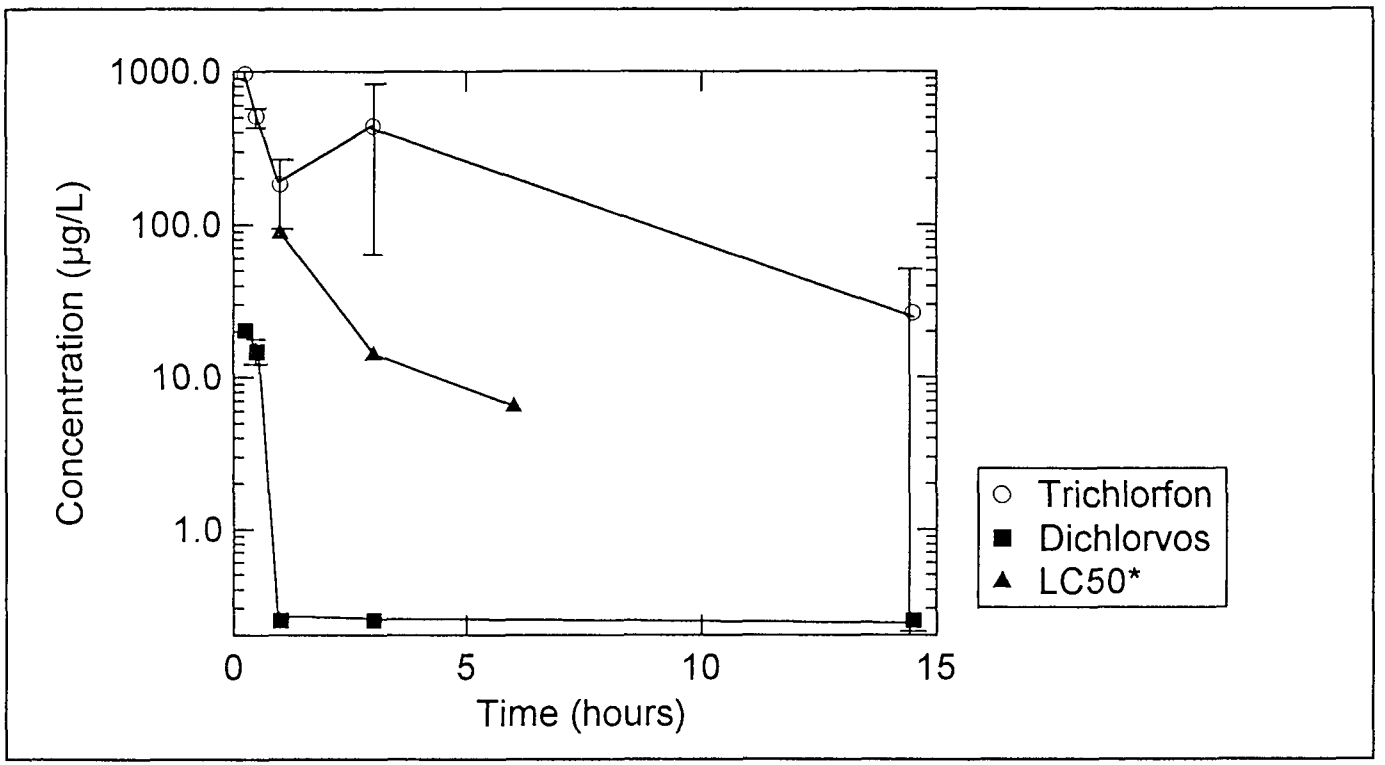


Figure 2b. Stream concentrations (arithmetic mean ± 1 SE) of trichlorfon and dichlorvos following an aerial application of trichlorfon (time=0 hours) in Block 215. Results of trichlorfon LC50 tests (*Daphnia carinata* at 25°C, for 1,3,6 h) are plotted for comparison.

* Nishiuchi, Y. 1979. Acute toxicity of pesticide formulations to *Daphnia carinata*. Suisan Zoshoku 27: 119-124 (in Japanese).

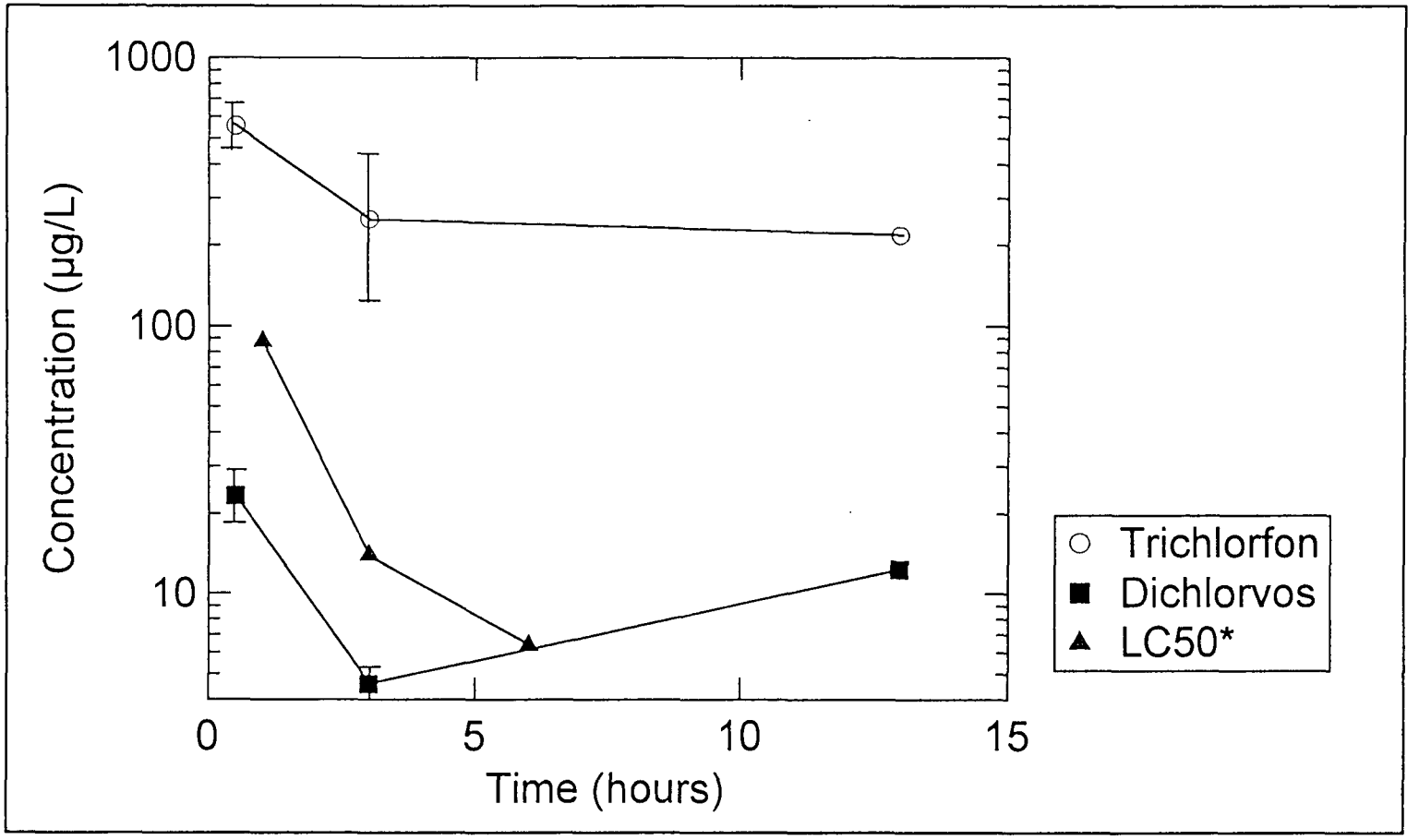


Figure 3a. Pond concentrations (arithmetic mean ± 1 SE) of trichlorfon and dichlorvos following an aerial application of trichlorfon (time=0 hours) in Block 213B. Results of trichlorfon LC50 tests (*Daphnia carinata* at 25°C, for 1,3,6 h) are plotted for comparison.

* Nishiuchi, Y. 1979. Acute toxicity of pesticide formulations to *Daphnia carinata*. Suisan Zoshoku 27: 119-124 (in Japanese).

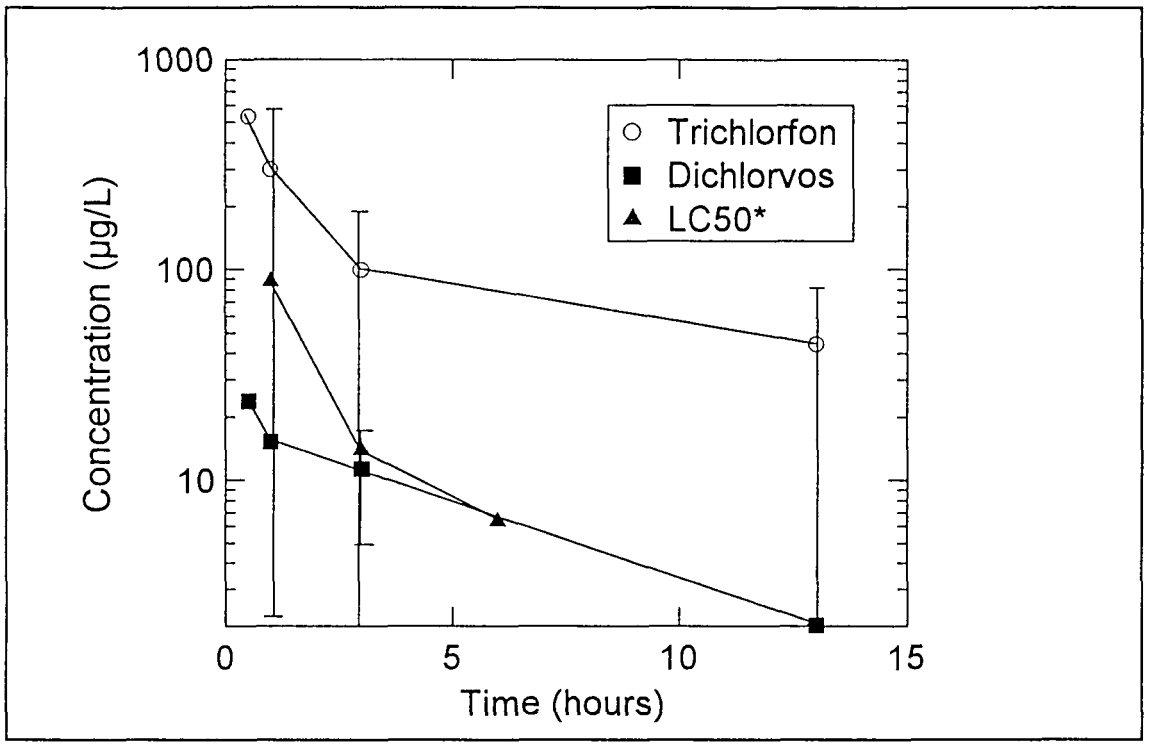


Figure 3b. Stream concentrations (arithmetic mean ± 1 SE) of trichlorfon and dichlorvos following an aerial application of trichlorfon (time=0 hours) in Block 213B. Results of trichlorfon LC50 tests (*Daphnia carinata* at 25°C, for 1,3,6 h) are plotted for comparison.

*Nishiuchi, Y. 1979. Acute toxicity of pesticide formulations to *Daphnia carinata*. Suisan Zoshoku 27: 119-124 (in Japanese).

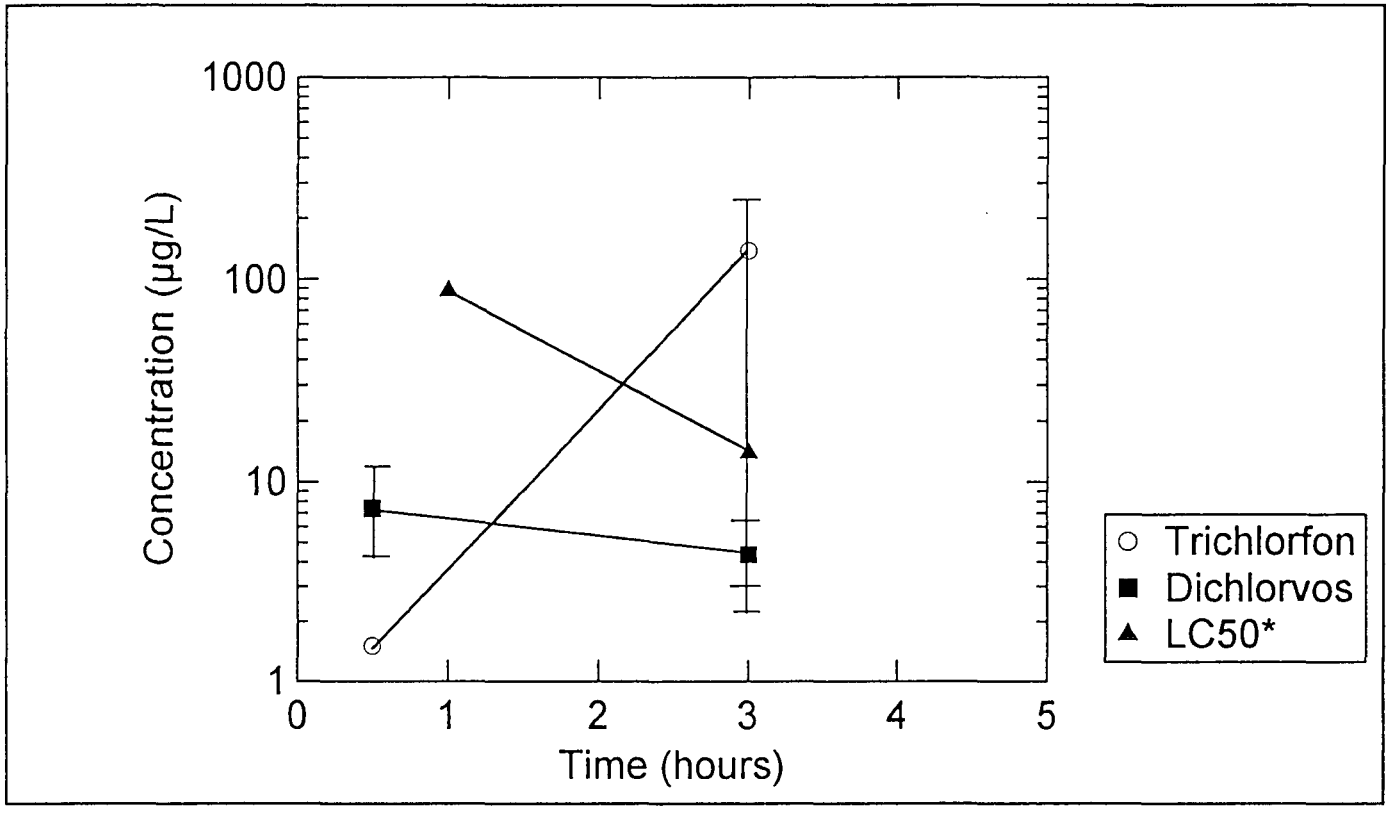


Figure 4a. Pond concentrations (arithmetic mean ± 1 SE) of trichlorfon and dichlorvos following an aerial application of trichlorfon (time=0 hours) in Block 213. Results of trichlorfon LC50 tests (*Daphnia carinata* at 25°C, for 1,3,6 h) are plotted for comparison.

*Nishiuchi, Y. 1979. Acute toxicity of pesticide formulations to *Daphnia carinata*. Suisan Zoshoku 27: 119-124 (in Japanese).

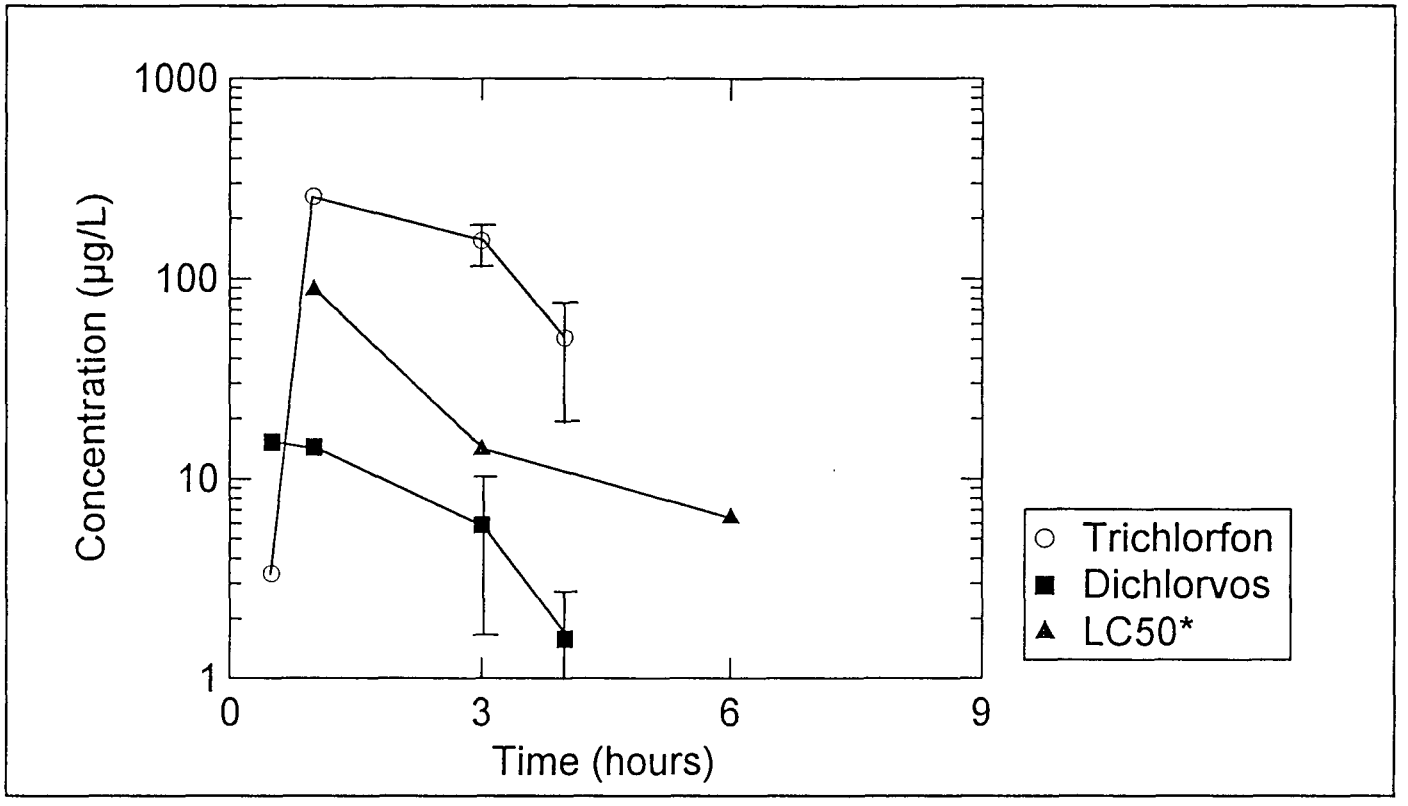
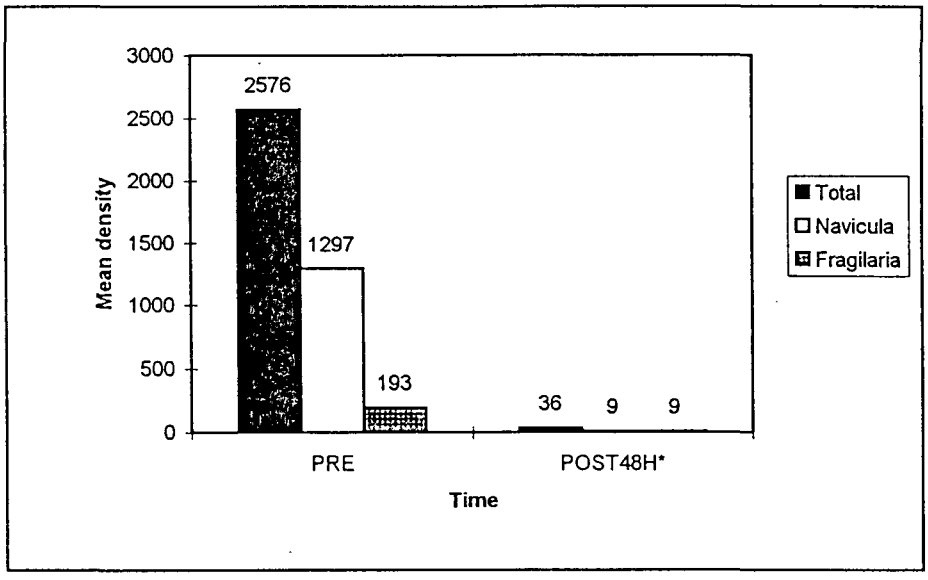


Figure 4b. Stream concentrations (arithmetic mean ± 1 SE) of trichlorfon and dichlorvos following an aerial application of trichlorfon (time=0 hours) in Block 213. Results of trichlorfon LC50 tests (*Daphnia carinata* at 25°C, for 1,3,6 h) are plotted for comparison.

*Nishiuchi, Y. 1979. Acute toxicity of pesticide formulations to *Daphnia carinata*. Suisan Zoshoku 27: 119-124 (in Japanese).



* Represents samples taken 48 h after initial spray event in Block 213 (24 h after spray event in Block 213B).

Figure 5. Block 213 phytoplankton enumeration.

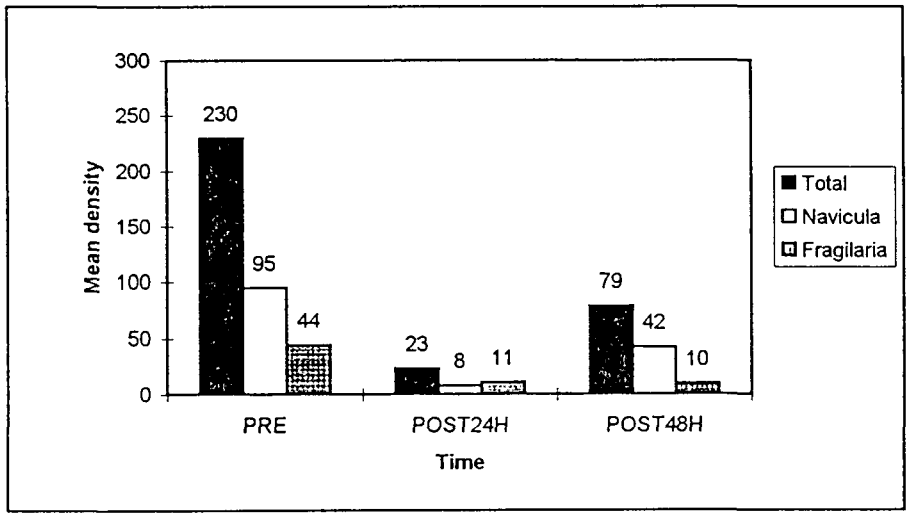
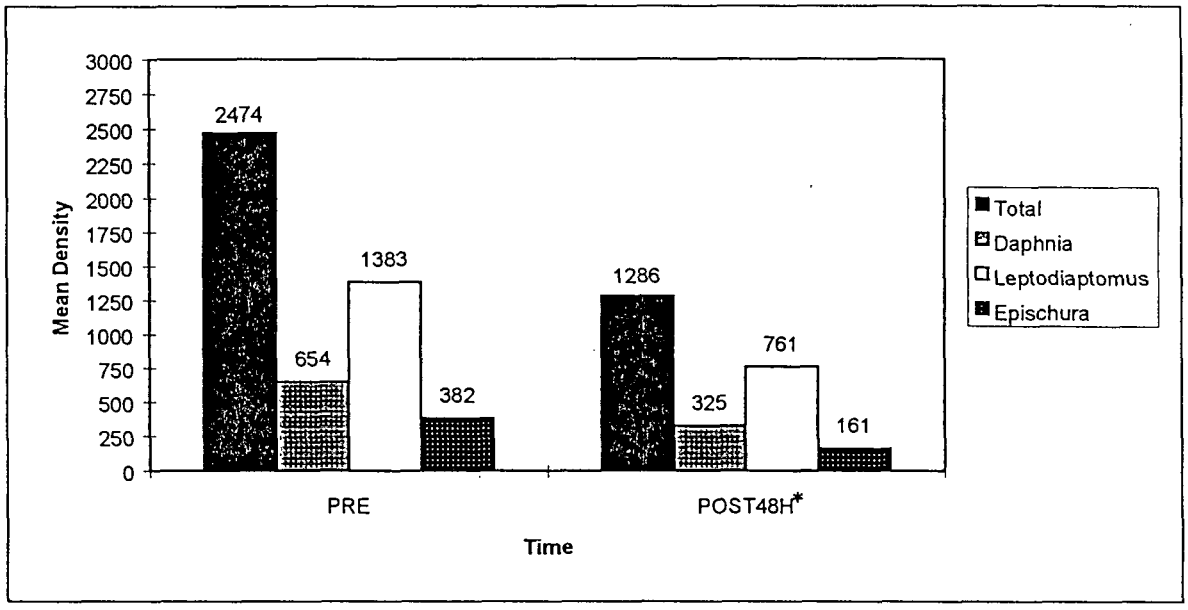


Figure 6. Block 215 phytoplankton enumeration.



* Represents samples taken 48 h after initial spray event in Block 213 (24 h after spray event in Block 213B)

Figure 7. Block 213 zooplankton enumeration.

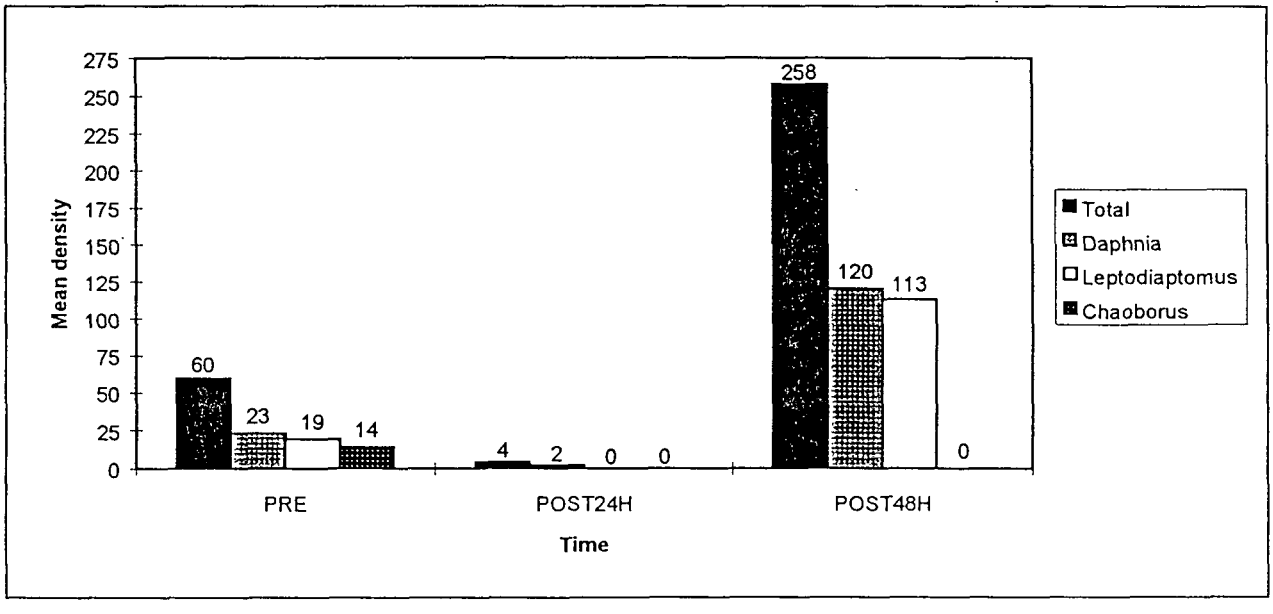
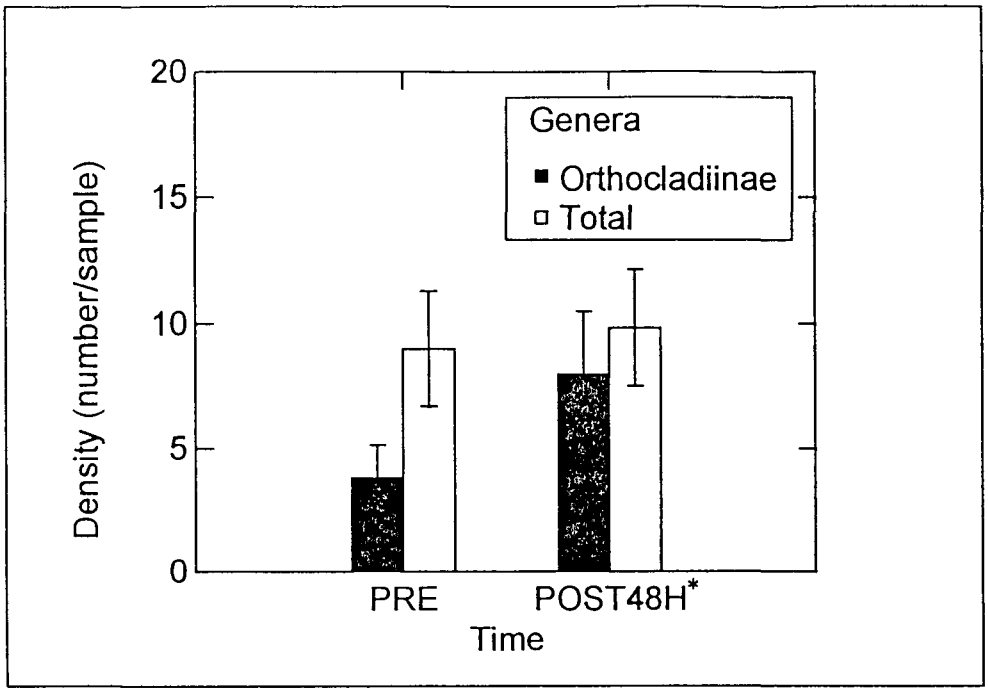


Figure 8. Block 215 zooplankton enumeration.



* Represents samples taken 48 h after initial spray event in Block 213 (24 h after spray event in Block 213B).

Figure 9. Density (arithmetic mean ± 1 SE) of stream macroinvertebrates before and after aerial application of trichlorfon in Block 213.

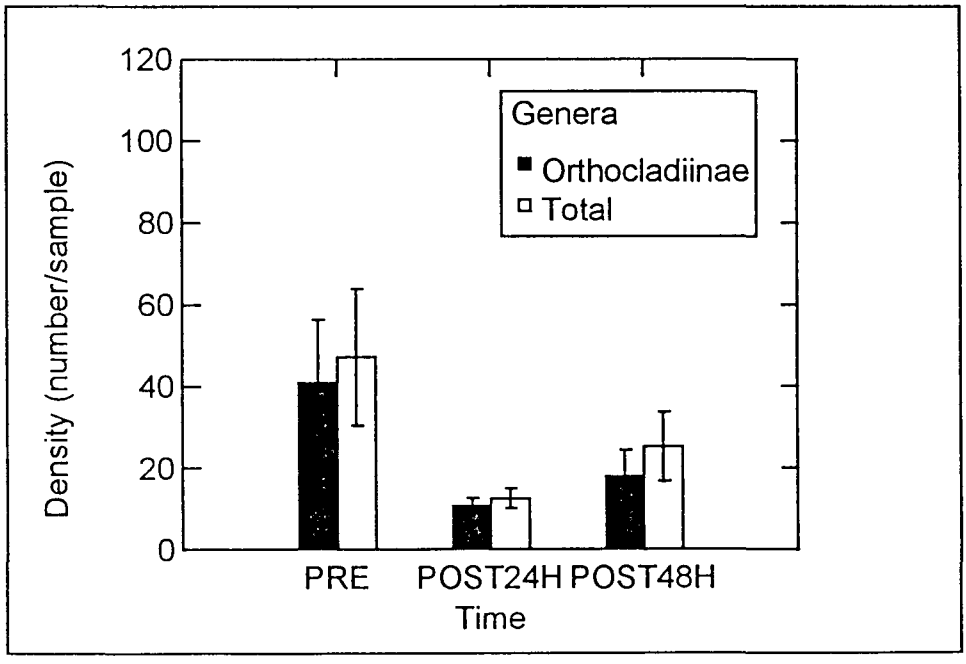


Figure 10. Density (arithmetic mean ± 1 SE) of stream macroinvertebrates before and after aerial application of trichlorfon in Block 215.

Table 1: Deposit filter concentrations

Date	Station	Sample	Trichlorfon (g/ha)	Dichlorvos (g/ha)
28-Jul-98	Block 213B	field blank	nd	nd
28-Jul-98	Block 213B	field blank	nd	nd
19-Jul-98	Block 215	filter blank	nd	nd
19-Jul-98	Block 215	empty filter blank	nd	nd
27-Jul-98	Block 215	pond filter post 30 mins.	5.12	nd
27-Jul-98	Block 215	pond filter post 30 mins.	37.48	nd
27-Jul-98	Block 215	pond filter post 30 mins.	4.46	nd
27-Jul-98	Block 215	pond filter post 30 mins.	0.96	nd
27-Jul-98	Block 215	pond filter post 30 mins.	0.92	nd
27-Jul-98	Block 215	pond filter post 30 mins.	0.44	nd
27-Jul-98	Block 215	pond filter post 30 mins.	0.36	3.13
27-Jul-98	Block 215	pond filter post 30 mins.	45.54	4.53
27-Jul-98	Block 215	pond filter post 30 mins.	nd	2.52
27-Jul-98	Block 215	pond filter post 30 mins.	0.90	nd
27-Jul-98	Block 215	stream filter post 30 mins.	4.46	2.78
27-Jul-98	Block 215	stream filter post 30 mins.	9.50	nd
27-Jul-98	Block 215	stream filter post 30 mins.	8.71	1.15
27-Jul-98	Block 215	stream filter post 30 mins.	1.81	3.06
27-Jul-98	Block 215	stream filter post 30 mins.	35.56	1.43
27-Jul-98	Block 215	stream filter post 30 mins.	2.57	2.48
27-Jul-98	Block 213	pond filter post	1.43	3.41
27-Jul-98	Block 213	pond filter post	2.41	nd
27-Jul-98	Block 213	pond filter post	1.14	nd
27-Jul-98	Block 213	pond filter post	0.78	nd
27-Jul-98	Block 213	pond filter post	0.97	0.48
27-Jul-98	Block 213	pond filter post	0.64	2.23
27-Jul-98	Block 213	pond filter post	1.73	nd
27-Jul-98	Block 213	pond filter post	0.88	1.91
27-Jul-98	Block 213	pond filter post	1.99	nd
27-Jul-98	Block 213	pond filter post	1.35	2.78
27-Jul-98	Block 213	stream filter post	0.90	2.25
27-Jul-98	Block 213	stream filter post	nd	nd
27-Jul-98	Block 213	stream filter post	9.14	nd
27-Jul-98	Block 213	stream filter post	nd	nd
27-Jul-98	Block 213	stream filter post	1.49	3.34
28-Jul-98	Block 213B	pond filter post	nd	3.06
28-Jul-98	Block 213B	pond filter post	nd	3.42
28-Jul-98	Block 213B	pond filter post	nd	nd
28-Jul-98	Block 213B	pond filter post	nd	nd
28-Jul-98	Block 213B	pond filter post	nd	2.99
28-Jul-98	Block 213B	pond filter post	5.55	1.51
28-Jul-98	Block 213B	pond filter post	nd	3.88
28-Jul-98	Block 213B	pond filter post	nd	3.26
28-Jul-98	Block 213B	pond filter post	nd	1.50
28-Jul-98	Block 213B	pond filter post	1.58	nd
28-Jul-98	Block 213B	stream filter post	3.64	2.32
28-Jul-98	Block 213B	stream filter post	nd	3.33
28-Jul-98	Block 213B	stream filter post	nd	0.93
28-Jul-98	Block 213B	stream filter post	18.76	nd
28-Jul-98	Block 213B	stream filter post	0.57	1.95
28-Jul-98	Block 213B	stream filter post	nd	2.79
28-Jul-98	Block 213B	stream filter post	nd	nd

Table 2: Trichlorfon and dichlorvos concentrations in water from ponds and streams in Dylox® spray blocks

Date	Station	Sample	Trichlorfon (µg/L)	Std dev.	Dichlorovos (µg/L)	Std dev.
19-Jul-98	Block 215	surface pond prespray	nd	nd	nd	nd
19-Jul-98	Block 215	subsurface pond prespray	nd	nd	0.57/nd	0.12
19-Jul-98	Block 215	stream prespray	nd	nd	nd	nd
19-Jul-98	Block 215	stream prespray	nd	nd	nd	nd
19-Jul-98	Block 213	pond prespray	nd	nd	nd	nd
19-Jul-98	Block 213	stream prespray	29.73/>8.0	1.56	nd	nd
27-Jul-98	Block 215	pond post 30 mins.	205.93	3.67	nd	nd
27-Jul-98	Block 215	pond post 30 mins.	459.72	3.89	nd	nd
27-Jul-98	Block 215	pond post 30 mins.	1053.7	12.13	20.44	2.27
27-Jul-98	Block 215	pond post 30 mins.	nd	nd	26.49	2.66
27-Jul-98	Block 215	pond post 30 mins.	nd	nd	40.77	3.43
27-Jul-98	Block 215	pond post 30 mins.	344.28	9.87	21.5	2.96
27-Jul-98	Block 215	pond post 3 hrs.	1124.74	10.23	nd	nd
27-Jul-98	Block 215	pond mid-depth post 3 hrs.	303.49	12.35	24.43	2.6
27-Jul-98	Block 215	pond post 14.5 hrs.	261.81	2.26	nd	nd
27-Jul-98	Block 215	pond mid-depth post 14.5 hrs.	325.11	3.44	nd	nd
27-Jul-98	Block 215	stream post 15 mins.	965.01	17.23	20.3	2.09
27-Jul-98	Block 215	stream post 30 mins.	465.09	13.56	13.97	1.42
27-Jul-98	Block 215	stream post 30 mins.	548.62	1.89	15.46	2.02
27-Jul-98	Block 215	stream post 1 hr.	277.42	9.57	nd	nd
27-Jul-98	Block 215	stream post 1 hr.	88.67	1.42	nd	nd
27-Jul-98	Block 215	stream post 3 hrs.	58.47	1.35	nd	nd
27-Jul-98	Block 215	stream post 3 hrs.	817.86	16.98	nd	nd
27-Jul-98	Block 215	stream post 14.5 hrs.	nd	nd	nd	nd
27-Jul-98	Block 215	stream post 14.5 hrs.	52.67	1.93	nd	nd
27-Jul-98	Block 213	pond post 30 mins.	nd	nd	nd	nd
27-Jul-98	Block 213	pond post 30 mins.	nd	nd	15.16	2.23
27-Jul-98	Block 213	pond post 30 mins.	nd	nd	14.44	2.04
27-Jul-98	Block 213	pond post 30 mins.	nd	nd	nd	nd
27-Jul-98	Block 213	pond post 3 hrs.	408.31	1.44	1.18	0.13
27-Jul-98	Block 213	pond post 3 hrs.	2.14	0.84	10.29	1.46
27-Jul-98	Block 213	pond post 3 hrs.	2.5	0.96	1.59	0.19
27-Jul-98	Block 213	pond mid-depth post 3 hrs.	nd	nd	10.53	2.22
27-Jul-98	Block 213	pond mid-depth post 3 hrs.	4.76	0.89	nd	nd
27-Jul-98	Block 213	pond mid-depth post 3 hrs.	242.6	2.52	6.71	0.61
27-Jul-98	Block 213	pond mid-depth post 3 hrs.	nd	nd	13.52	2.28
27-Jul-98	Block 213	stream post 30 mins.	3.35	0.84	15.27	2.42
27-Jul-98	Block 213	stream post 1 hr.	256.03	1.84	14.38	1.31
27-Jul-98	Block 213	stream post 3 hrs.	128.61	1.57	1.25	0.16
27-Jul-98	Block 213	stream post 3 hrs.	182.53	1.43	10.57	1.5
27-Jul-98	Block 213	stream post 4 hrs.	nd	nd	nd	nd
27-Jul-98	Block 213	stream post 4 hrs.	51.34	1.25	4.74	0.83
27-Jul-98	Block 213	stream post 4 hrs.	101.59	1.69	nd	nd
28-Jul-98	Block 213B	pond post 30 mins.	686.15	4.63	38.74	1.61
28-Jul-98	Block 213B	pond post 30 mins.	569.83	10.93	15.6	1.92
28-Jul-98	Block 213B	pond post 30 mins.	784.7	18.97	15.18	1.27

Date	Station	Sample	Trichlorfon (µg/L)	Std dev.	Dichlorovos (µg/L)	Std dev.
28-Jul-98	Block 213B	pond post 30 mins.	**430.23**	13.67	**13.92**	1.37
28-Jul-98	Block 213B	pond post 30 mins.	**227.1**	11.88	**17.66**	2.1
28-Jul-98	Block 213B	pond post 30 mins.	**631.24**	12.3	**38.89**	1.97
28-Jul-98	Block 213B	pond post 3 hrs.	**531.67**	12.52	nd	nd
28-Jul-98	Block 213B	pond post 3 hrs.	nd	nd	**10.17**	1.39
28-Jul-98	Block 213B	pond post 3 hrs.	**84.29**	1.34	nd	nd
28-Jul-98	Block 213B	pond post 3 hrs.	**384.85**	8.76	**8.08**	0.78
28-Jul-98	Block 213B	pond mid-depth post 3 hrs.	**903.99**	17.21	**7.91**	1.34
28-Jul-98	Block 213B	pond mid-depth post 3 hrs.	nd	nd	**12.84**	1.75
28-Jul-98	Block 213B	pond post 13 hrs.	**216.98**	2.55	**12.32**	1.48
28-Jul-98	Block 213B	pond mid-depth post 13 hrs.	nd	nd	nd	nd
28-Jul-98	Block 213B	stream post 30 mins.	**533.88**	12.12	**23.81**	2.18
28-Jul-98	Block 213B	stream post 1 hr.	**7.05**	1.02	**14.59**	2.55
28-Jul-98	Block 213B	stream post 1 hr.	**592.24**	11.59	**15.95**	1.44
28-Jul-98	Block 213B	stream post 3 hrs.	**299.46**	10.99	nd	nd
28-Jul-98	Block 213B	stream post 3 hrs.	nd	nd	**12.11**	1.33
28-Jul-98	Block 213B	stream post 3 hrs.	nd	nd	**22.01**	1.73
28-Jul-98	Block 213B	stream post 13 hrs.	**88.43**	0.63	**1.44**	0.13
28-Jul-98	Block 213B	stream post 13 hrs.	nd	nd	**2.61**	0.27
28-Jul-98		dichloromethane blank	nd	nd	nd	nd

Table 3: Acute Toxicity of Trichlorfon for non-target aquatic organisms

Fish	pH	temp.	1-hr LC50 (µg/L)	3-hr LC50 (µg/L)	6-hr LC50 (µg/L)	24-hr LC50 (µg/L)	48-hr LC50 (µg/L)	96-hr LC50 (µg/L)
Oncorhynchus mykiss - Rainbow trout[3]	6.5	7°C						40900
Oncorhynchus mykiss - Rainbow trout[3]	6.5	17°C						2500
Oncorhynchus mykiss - Rainbow trout[3]	9.5	7°C						520
Oncorhynchus mykiss - Rainbow trout[3]	9.5	17°C						330
Oncorhynchus mykiss - Rainbow trout[7]							3200	
Salvelinus fontinalis - Brook trout[4]	6.5-9.0					340 - >12000		240 - 9400
Salvelinus namaycush - Lake trout[4]	7.4					2680 - 6150		550 - 1030
Salmo clarki - Cutthroat trout[9]								375
Salmo salar - Atlantic salmon[4]	6.5-8.5					2000 - >12000		300 - 4400
Cyprinodon variegatus - Sheepshead minnows[1]		17°C						18700 (at 30 ppt salinity)
Cyprinodon variegatus - Sheepshead minnows[1]		17°C						19300 ug/L (at 15 ppt salinity)
Morone saxatilis - striped bass fingerlings[2]								5200
Morone saxatilis - striped bass[7]						10400		
Lepomis macrochirus - Bluegill[2]								3800 (in soft water)
Pimephales promelas - Fathead minnow[2]								51 (in hard water)
Pimephales promelas - Fathead minnow[2]								180000 (in soft water)
Pimephales promelas - Fathead minnow[7]								180000 (in soft water)
Aquatic Invertebrates	**pH**	**temp.**	**1-hr LC50 (µg/L)**	**3-hr LC50 (µg/L)**	**6-hr LC50 (µg/L)**	**24-hr LC50 (µg/L)**	**48-hr LC50 (µg/L)**	**96-hr LC50 (µg/L)**
Gammarus pseudolimnaeus - Amphipod[3]	6.5	7°C						11000
Gammarus pseudolimnaeus - Amphipod[3]	6.5	17°C						140
Gammarus pseudolimnaeus - Amphipod[3]	9.5	7°C						70
Gammarus pseudolimnaeus - Amphipod[3]	9.5	17°C						20
Gammarus pseudolimnaeus - Amphipod[4]	7.1 - 8.5					75 - 660		17 - 275
Gammarus pseudolimnaeus - Amphipod[9]	7.5	12°C						108
Gammarus pseudolimnaeus - Amphipod[9]	8.5	12°C						52
Palaemonetes spp. - Grass shrimp[1]		17°C						7.1 (at 30ppt salinity)
Palaemonetes spp. - Grass shrimp[1]		17°C						11.3 (at 15 ppt salinity)
Pteronarcella badia - Stonefly[9]	6.5	12°C				50 - 320		5.3
Pteronarcella badia - Stonefly[9]	7.5	12°C						9.8
Pteronarcella badia - Stonefly[9]	8.5	12°C						100
Daphnia - Water flea[8]							0.96 (48 h EC50)	
Daphnia carinata - Water flea[5]		25°C	88	14	6.4	1.2	0.75	
Cloeon diptrum - Mayfly[6]		25°C		1800	750	75	56	

Chronic toxicity testing with aquatic invertebrates indicate that the Maximum Allowable Toxicant Concentration (MATC) for trichlorfon is between 5.6 and 8.6 ng/L. The MATC for fish is between 110 and 160 ug/L.*
* United States Environmental Protection Agency, R.E.D. Facts, Trichlorfon, EPA-738-F-98-017, January 1997

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Table 4: Toxicity of water from ponds and streams in Dylox® spray blocks to *Daphnia magna* (48 h exposures)

Sample ID	Percentage Immobilized	Percentage Dead
Control 1	0	0
Control 2	0	0
Control 3	0	0
Control 4	0	0
Control 5	0	0
213, pre-spray pond July 20, 1998	0	0
213, pre-spray pond July 20, 1998 hardness unadjusted (hardness 10.8 mg/L)	0	0
213, 0.5 hour July 27, 1998	0	0
213, 1 hour July 27, 1998	0	0
213, 2 hours July 27, 1998	0	0
213, 3 hours July 27, 1998	0	0
213, 4 hours July 27, 1998	0	0
213B, 0.5 hour July 28, 1998	0	0
213B, 1 hour July 28, 1998	0	0
213B, 2 hours July 28, 1998	100	0
213B, 3 hours July 28, 1998	100	0
215, surface pre-spray control pond July 19, 1998	0	0
215, surface pre-spray control pond July 19, 1998 hardness unadjusted (hardness 11.0 mg/L)	0	0
215, subsurface pre-spray control July 19, 1998	0	0
215, subsurface pre-spray control July 19, 1998 hardness unadjusted (hardness 9.6 mg/L)	0	0
215, 15 min. July 27, 1998	100	0
215, 30 min. July 27, 1998	100	10
215, 1 hour July 27, 1998	100	80
215, 2 hours July 27, 1998	100	10
215, 3 hours July 27, 1998	100	30
215, 4 hours July 27, 1998	100	0
215, 5 hours July 27, 1998	100	0

Table 5: Phytoplankton identification and enumeration in water samples taken from Dylox® spray blocks

Phylum	Class	Order	Suborder	Family	Genus	Block 213 pre-spray[1]	Block 213 post 48h[2]	Block 215 pre-spray	Block 215 post 23h	Block 215 post 48h
						AVG/sample	AVG/sample	AVG/sample	AVG/sample	AVG/sample
Chrysophyta	Chysophyceeae	Chrysomonadales	-	Mallomonadales	*Mallomonas*	**12**	1	**1**		
Chrysophyta	Bacillariophyceae	Centrales	Conscinodiscineae	Coscinodiscaceae	*Melosira*	**84**		**3**		
Chrysophyta	Bacillariophyceae	Centrales	Conscinodiscineae	Coscinodiscaceae	*Stephanodiscus*	**7**	1	**2**		
Chrysophyta	Bacillariophyceae	Centrales	Biddulphineae	Chaaetoceraceae	*Chaetoceros*			**1**		
Chrysophyta	Bacillariophyceae	Pennales	Fragilarineae	Tabellariaceae	*Tabilleria*	**27**	1	**4**		**5**
Chrysophyta	Bacillariophyceae	Pennales	Fragilarineae	Meridionaceae	*Meridion*	**4**	2	**3**		
Chrysophyta	Bacillariophyceae	Pennales	Fragilarineae	Diatomaceae	*Diatoma*	**22**	2	**1**		
Chrysophyta	Bacillariophyceae	Pennales	Fragilarineae	Diatomaceae	*Opephora*			**1**		
Chrysophyta	Bacillariophyceae	Pennales	Fragilarineae	Fragilariaceae	*Fragilaria*	**193**	9	**44**	11	10
Chrysophyta	Bacillariophyceae	Pennales	Fragilarineae	Fragilariaceae	*Synedra*	**19**		**1**		
Chrysophyta	Bacillariophyceae	Pennales	Fragilarineae	Eunotiaceae	*Eunotia*	**99**	1	**8**	1	
Chrysophyta	Bacillariophyceae	Pennales	Fragilarineae	Eunotiaceae	*Amphicampa*			**2**		
Chrysophyta	Bacillariophyceae	Pennales	Achnanthineae	Achnanthaceae	*Achnanthes*	**102**	1	**4**		
Chrysophyta	Bacillariophyceae	Pennales	Achnanthineae	Achnanthaceae	*Cocconeis*	**7**		**1**		
Chrysophyta	Bacillariophyceae	Pennales	Naviculineae	Naviculaceae	*Navicula*	**1297**	9	**95**	8	42
Chrysophyta	Bacillariophyceae	Pennales	Naviculineae	Naviculaceae	*Pinnularia*	**95**	1	**7**		3
Chrysophyta	Bacillariophyceae	Pennales	Naviculineae	Naviculaceae	*Diploneis*	**8**				
Chrysophyta	Bacillariophyceae	Pennales	Naviculineae	Naviculaceae	*Stauroneis*	**97**	3	**10**	1	5
Chrysophyta	Bacillariophyceae	Pennales	Naviculineae	Naviculaceae	*Amphipleura*	**14**				**6**
Chrysophyta	Bacillariophyceae	Pennales	Naviculineae	Naviculaceae	*Frustulia*	**125**	2	**9**		1
Chrysophyta	Bacillariophyceae	Pennales	Naviculineae	Gomphonemataceae	*Gomphonema*	**19**		**7**	1	2
Chrysophyta	Bacillariophyceae	Pennales	Naviculineae	Cymbellaceae	*Cymbella*	**104**	1	**19**	1	2
Chrysophyta	Bacillariophyceae	Pennales	Naviculineae	Cymbellaceae	*Amphora*	**180**	3	**6**		1
Chrysophyta	Bacillariophyceae	Pennales	Naviculineae	Cymbellaceae	*Epithemia*				1	**1**
Chrysophyta	Bacillariophyceae	Pennales	Surirellineae	Nitzschiaceae	*Nitzschia*	**38**	1	**1**		
Chrysophyta	Bacillariophyceae	Pennales	Surirellineae	Nitzschiaceae	*Denticula*	**23**		**2**	1	
Chrysophyta	Bacillariophyceae	Pennales	Surirellineae	Surirellaceae	*Surirella*	**4**				**1**
Chlorophyta	Chlorophyceae	Zygnematales	-	*Desmidiaceae*	*Closterium*		**1**			
Chlorophyta	Chlorophyceae	Zygnematales	-	Desmidiaceae	*Spinoclosterium*			**1**		
Chrysophyta	Chlorophyceae	Zygnematales	-	Desmidiaceae	*Cosmarium*		**1**	**2**		
Chrysophyta	Chlorophyceae	Zygnematales	-	Desmidiaceae	*Staurustrum*	**2**	1			**1**
Cyanophyta	Myxophyceae	Oscillatoriales	Oscillatorineae	Oscillatoriaceae	*Spirulina*					**1**
Mean Total No. of Individuals / Sample						**2578**	36	**230**	23	79

Sample volume = 5 ml

[1] numbers calibrated to 5ml volume

[2] values represent mean density (no./sample) of phytoplankton 48 h after the initial spray in Block 213, which was also 24 h after the subsequent spray in Block 213B.
bolded values indicate the higher number of individuals per sample comparing pre-spray and post-spray numbers

Table 6: Zooplankton identification and enumeration in water samples taken from Dylox® spray blocks

Phylum	Class	Subclass	Order	Family	Genus	Block 213 pre-spray	Block 213 post 48h[1]	Block 215 pre-spray	Block 215 post 23h	Block 215 post 48h
						AVG/sample	AVG/sample	AVG/sample	AVG/sample	AVG/sample
Arthropoda	Crustacea	Branchiopoda	Cladocera	Daphnidae	*Daphnia*	**654**	325	23	2	**120**
Arthropoda	Crustacea	Branchiopoda	Cladocera	Daphnidae	*Ceriodaphnia*	32	32			**7**
Arthropoda	Crustacea	Branchiopoda	Cladocera	Sididae	*Latona*	**2**				
Arthropoda	Crustacea	Branchiopoda	Cladocera	Sididae	*Sida*		**4**			**2**
Arthropoda	Crustacea	Branchiopoda	Cladocera	Bosminidae	*Eubosmina*				**2**	
Arthropoda	Crustacea	Copepoda	Calanoida	Diaptomidae	*Leptodiaptomus*	**1383**	761	20		**113**
Arthropoda	Crustacea	Copepoda	Calanoida	Temoridae	*Epischura*	**382**	161	2		**16**
Arthropoda	Crustacea	Copepoda	Cyclopoida	Cyclopidae	*Cyclops*	**21**	4	**2**		
Arthropoda	Insecta	-	Diptera	Chaoboridae	*Chaoborus*			**14**		
Mean Total No. of Individuals / Sample						**2474**	1286	60	4	**258**

Sample volume = 1 m^3

[1] values represent mean density (no./sample) of zooplankton 48 h after the initial spray in Block 213, which was also 24 h after the subsequent spray in Block 213B.
bolded values indicate the higher number of individuals per sample comparing pre-spray and post-spray numbers

Table 7: Aquatic invertebrates from Surber and drift samples obtained from the stream in Block 213.

			SURBER		DRIFT																
		APPLICATION	PRE-SPRAY (July 20)	POST-(48H) * (July 29)	PRE-(4:50-5:10 PM)	POST- (7:25 AM)	POST-(7:55 AM)	POST-(8:55 AM)	POST-(9:55 AM)	POST-(10:55 AM)	PRE-(9:30 PM)	POST-(9:30 PM)	PRE-(10:30 PM)	POST-(10:30 PM)	PRE-(11:30 PM)	POST-(11:30 PM)	PRE-(3:30 AM)	POST-(3:30 AM)	PRE-(4:30 AM)	POST-(4:30 AM)
Order	**Family**	**Genus**																			
Coleoptera	Dytiscidae	Agabus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Collembola	Isotomus	Isotomus	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Diptera	Chironomidae	Orthocladiinae	23	48	0	0	0	0	0	0	1	0	1	0	1	2	0	0	1	0	
Diptera	Chironomidae	Tanypodinae	7	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
Diptera	Simuliidae	Simulia	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
Ephemeroptera	Baetidae	Baetis	4	2	0	0	0	0	0	0	0	0	3	0	0	0	2	0	0	0	
Ephemeroptera	Ephemerellidae	Ephemerella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Plecoptera	Chloroperlidae	Alloperla	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Plecoptera	Leuctridae	Leuctra	15	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Trichoptera	Limnephilidae	Psychoglypha	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Trichoptera	Rhyacophilidae	Rhyacophila	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Total number of individuals counted in all samples			54	59	0	0	0	0	0	0	2	0	5	1	2	2	2	0	1	0	

* Samples were taken 48 h after the initial spray event on Block 213, which was also 24 h after the second spray event on Block 213B.

Table 8: Aquatic invertebrates from Surber and drift samples obtained from the stream in Block 215.

			SURBER				DRIFT																	
		APPLICATION	PRE-SPRAY (July 20)	POST-(24H) (July 28)	POST-(48H) (July 29)		PRE-(5:22-5:37 PM)	PRE- (6:45 PM)	PRE-(7:45 PM)	POST-(30 MIN.)	POST-(1H)	POST-(2H)	POST-(3H)	POST-(4H)		PRE-(9:45-10:00 PM)	POST-(9:30-9:45 PM)	PRE-(10:50-11:05 PM)	POST-(10:30-10:45 PM)	PRE-(11:45 PM-12:00 AM)	POST-(11:30-11:45 PM)	PRE-(3:45-4:00 AM)	POST-(3:30-3:45 AM)	PRE-(4:45-5:00 AM)	POST-(4:30-4:45 AM)
Order	**Family**	**Genus**																						
Coleoptera	Dytiscidae	Agabus	1	0	1		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0
Collembola	Isotomus	Isotomus	0	1	4		0	5	0	0	0	1	0	0		0	0	0	0	0	0	0	0	5	1
Diptera	Chironomidae	Orthocladiinae	246	64	108		2	4	3	1	1	0	1	0		4	0	2	2	0	2	0	1	3	1
Diptera	Chironomidae	Tanypodinae	12	7	16		0	1	0	0	0	0	0	0		0	0	0	0	0	0	0	0	1	0
Diptera	Simuliidae	Simulia	3	0	2		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0
Ephemeroptera	Baetidae	Baetis	7	0	6		0	1	0	0	0	0	0	0		0	0	0	1	0	1	0	0	0	0
Ephemeroptera	Ephemerellidae	Ephemerella	1	0	5		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0
Plecoptera	Chloroperlidae	Alloperla	0	0	1		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0
Plecoptera	Leuctridae	Leuctra	5	0	6		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0
Trichoptera	Limnephilidae	Psychoglypha	0	3	2		0	0	1	1	0	0	0	0		0	1	0	0	0	1	0	0	0	2
Trichoptera	Rhyacophilidae	Rhyacophila	2	0	0		0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0
Total number of individuals counted in all samples			277	75	151		2	11	4	2	1	1	1	0		4	1	2	3	0	4	0	1	9	4